Persistence of Circulating Complexes between HBsAg and Immunoglobulin M in Sera of Hepatitis B Surface Antigen Positive Patients Suffering from Liver Cirrhosis or Primary Liver Cancer

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ABSTRACT

Complexes between hepatitis B surface antigen (HBsAg) and immunoglobulin M (IgM) have been detected in acute type B hepatitis. Sequential serum testing for the presence of these complexes has been shown to be the best method for predicting disease chronicity.

The presence of HBsAg/IgM complexes was investigated using an enzyme-linked immunosorbent assay with selected sera from Senegal. The three population groups studied were composed of 405 Senegalese soldiers as well as 84 liver cirrhosis and 169 primary liver cancer patients. Only one of the 122 HBsAg negative sera tested was found to be positive for HBsAg/IgM complexes. Complexes were detected 13.9% of the HBsAg positive soldiers, in 40% of the HBsAg positive liver cirrhosis patients, and in 50% of the HBsAg positive primary liver cancer patients. HBsAg/IgM complexes were also detected in 53.6% of the hepatitis B e antigen (HBe) positive soldiers, compared to 75 and 76% for the HBeAg positive liver cirrhosis and primary liver cancer patients, respectively. In anti-HBe positive sera, an increased proportion of HBsAg/IgM complexes was observed during the sequence chronic hepatitis (5%)-cirrhosis (29%)-primary liver cancer (42%). On the other hand, it has been reported that in the sequence of events leading from chronic hepatitis to primary liver cancer, there is an increase in anti-HBeAg prevalence and in α-fetoprotein levels. In this study, only α-fetoprotein levels were found to increase. Values higher than 15 IU/ml were observed in 4.3, 27.3, and 86.4% of the HBsAg positive individuals from the three groups. No significant variation was observed in the anti-HBe prevalence between the population group (64-75%).

INTRODUCTION

In Senegal, as in many African and Asian countries, hepatitis B infection occurs mainly during infancy and up to 90% of the adult population show evidence of past or present infection (1-3). In addition, 10 to 15% of the adult population are HBsAg carriers. In such countries, PLC happens to be one of the leading cancers, with HBV being implicated as the etiological agent of both LC and primary liver cancer (4-6). Final epidemiological evidence has come from studies of HBsAg carriers and noncarriers (7-9). Beasley et al. (8), in a study carried out in Taiwan, estimated that HBV carriers run over 200 times more risk of contracting PLC than do noncarriers.

For these reasons, it seems important to know whether there is any serological marker that could be used to predict in which HBsAg carriers cirrhosis or cancer is likely to develop. AFP and anti-HBe antibodies have been suggested to be such markers. In this respect, it has been reported that in the sequence of events leading from chronic hepatitis to LC and to PLC, there is an increase in anti-HBe prevalence and in AFP levels (9-13). However, in a previous study carried out in Senegal, no difference in HBsAg carrier state or in anti-HBe prevalence was observed between LC and PLC (14). On the other hand, screening for AFP must be performed often to detect, before the onset of clinical symptoms, which patients are developing a liver cancer.

Recently, complexes between HBsAg and IgM have been detected in acute type B hepatitis, and experimental results suggest that sequential serum testing for the presence of these complexes provides the best means of predicting the chronicity of the disease (15-17). Such complexes have also been detected in 13.9% of healthy HBsAg carriers from Senegal (18), showing that in some carriers these complexes persist for several years after infection which occurs during childhood (2).

In the present study, we looked for any such complexes in Senegalese patients suffering from either liver cirrhosis or primary liver cancer, in order to determine whether these complexes could be of any value as prognostic markers of progression towards these diseases.

MATERIALS AND METHODS

Serum samples were collected from soldiers of the Senegalese army (18), as well as hospital LC and PLC patients (Hôpital Le Dantec, Dakar, Senegal). HBsAg positive soldiers were screened by means of enzyme-linked immunosorbent assay (Behring), and HBsAg positive patients by means of radioimmunoassay (Austria II; Abbott Laboratories). LC and PLC diagnoses were based on clinical observations, liver ultrasonography evidence, and AFP levels.

HBsAg positive individuals (375 soldiers, 45 LC patients, and 116 PLC patients) were examined for anti-HBe, HBeAg, AFP, and HBsAg/IgM complexes. HBeAg and anti-HBe were detected by radioimmunoassay (Abbott HBe; Abbott Laboratories), and AFP was found with an enzyme-linked immunosorbent assay (kindly provided by Pasteur Diagnos-
Detection of the HBsAg/lgM Complexes. HBsAg/lgM complexes present in the samples were bound to anti-human IgM fixed in the wells of microplates (Cooke microtiter; Dynatech Microelisa). In the assay procedure, wells were incubated overnight at room temperature with 200 μl of 1/20 dilutions of test and control sera. After washing, 200 μl of peroxidase-conjugated anti-HBs were added, then incubated for 1 h at 40°C, and subsequently washed. Hydrogen peroxide in citrate-phosphate buffer solution (100 μl) was added. After 30 min incubation at room temperature 100 μl of dilute sulfuric acid were added, and the color intensity of the samples was measured photometrically against those of negative control sera using a Kontron SLT 210 photometer.

The conjugate was a monoclonal anti-HBs antibody of anti-"a" specificity (kindly provided by Dr. Lee of the Centre National de Transfusion Sanguine, Paris, France, and Dr. Pere of Pasteur Diagnostics). A 1/4000 diluted phosphate buffered solution containing 1% human albumin and 1% HBV negative human sera was used.

RESULTS

HBsAg/lgM complexes were tested for in HBsAg negative sera from 30 soldiers, 39 LC patients, and 53 PLC patients. Only one serum from a patient with cirrhosis showed evidence of the presence of HBsAg/lgM complexes.

HBsAg/lgM complexes were detected in 52 of the 375 HBsAg positive soldiers (13.9%) compared to 18 of 45 HBsAg positive cirrhosis patients (40.0%) and 58 of 116 HBsAg positive PLC patients (50.0%) (P < 10^-6) (Table 1). Anti-HBe antibody was detected in 282 (75.2%) chronic carriers (soldiers), in 28 (62.2%) cirrhosis patients, and in 72 (62.1%) PLC patients.

Quantities of sera obtained from some individuals were rather limited in volume; therefore it was possible to test for HBsAg in only 52 of the 93 anti-HBe negative soldiers, 16 of the 17 anti-HBe negative LC patients, and 38 of the 44 anti-HBe negative PLC patients.

HBsAg was found in 28 HBsAg chronic carriers, in 8 LC patients, and in 21 PLC patients. HBsAg/lgM complexes were detected in 53.6% of the HBsAg positive soldiers, compared to 75.0 and 76.0% for the HBsAg positive LC and PLC patients, respectively (Table 2) (P not significant).

In anti-HBe positive sera, an increased proportion of HBsAg/lgM complexes was observed in chronic carriers, 5%; in cirrhosis patients, 28.6%; and in PLC patients, 41.7% (P < 10^-4).

It has been reported that in the sequence of events leading from chronic hepatitis to cirrhosis and primary liver cancer, there is an increase in anti-HBe prevalence (9) and in AFP levels (13). In this study, abnormal AFP levels (>15 IU/ml) were found to increase from 4.3% in chronic carriers to 27.3% in LC patients and to 86.4% in PLC patients (Fig. 1).

DISCUSSION

In Senegal, HBV infection occurs during childhood (2); adults who are detected to be HBsAg carriers are therefore regarded as long term chronic carriers of hepatitis B virus. HBsAg/lgM complexes, which are a general feature of the early stage of HBV infection (16), persist in some cases for a very long period of time. These were detected in 13.9% of healthy chronic carriers, and in 40 and 50% of both HBsAg positive cirrhosis and primary liver cancer patients. As observed in acute hepatitis (17), HBsAg/lgM complexes are more often detected in HBsAg-positive sera than in anti-HBe positive sera. The relative rates of detection of HBsAg/lgM complexes in HBsAg and anti-HBe positive sera vary from 10.7 in healthy chronic carriers, to 2.6 in LC, and to 1.8 in PLC patients. Furthermore, an increase in the proportion of complexes was observed in both HBsAg and anti-HBe positive sera during the progression from chronic hepatitis to cirrhosis, and then to primary liver cancer. However, no significant variation in the prevalence of anti-HBe between the three population groups has observed in other studies.

These results seem to indicate that carriers of HBsAg are more likely to develop LC or PLC when their sera show evidence of the presence of HBsAg/lgM complexes. This hypothesis is reinforced by the fact that high AFP levels are more often observed in healthy HBsAg carriers in whose sera there is...
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In conclusion, it should be noted that in areas where both HBV infection and PLC are endemic, it is of great importance to select HBsAg carriers and then examine them for eventual appearance of cirrhosis or liver cancer. The screening of such a population for HBsAg/IgM complexes should lead to the selection of a group with a very high risk of evolution towards cirrhosis or primary liver cancer. With such patients, AFP levels determinations as well as ultrasonography examinations of the liver (19) must be regularly performed.

REFERENCES

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